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Atherogenic Index of Plasma, Castelli Risk Indexes and Leptin/Adiponectin Ratio in Women with Metabolic Syndrome

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Abstract: ***The aim of the study was to assess the lipid atherogenic risk in women with metabolic syndrome (MS) based on calculating the atherogenic index of plasma (AIP) {(logTriglycerides)/HDL-cholesterol}, Castelli's I and II risk indexes (the ratios total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol, respectively) and the leptin/adiponectin ratio (L/A ratio).***
Materials and methods: The study comprised of 31 women with MS (mean age 29.03±6.16 years) and 30 age-matched, clinically healthy women (mean age 26.33±4.49 years, P>0.05) forming a control group. The following clinical measurements and laboratory tests were performed on all participants: weight, height, waist circumference, hip circumference, systolic and diastolic blood pressure (SBP and DBP), fasting plasma glucose (FPG), fasting immunoreactive insulin (IRI), total cholesterol (TC), HDL-cholesterol (HDL-C) and triglycerides (TG). The well-known adipocytokines - leptin and adiponectin were determined. Body mass index (BMI), waist-hip ratio (WHR), homeostasis model assessment insulin resistance index (HOMA index), LDL-cholesterol (LDL-C), AIP, Castelli' s I and II risk indexes and the L/A ratio were calculated.
Results: The women with MS had significantly higher weight, BMI, waist circumference, hip circumference, IRI, HOMA index, TG, leptin, SBP and DBP compared to the healthy women, while WHR, FPG, TC, LDL-C and adiponectin were similar in both of the studied groups. AIP (-0.006±0.32 vs. -0.212±0.26, P=0.008), Castelli's risk index I (4.39±1.21 vs. 3.64±1.07, P=0.01) and Castelli's risk index II (2.82±1.01 vs. 2.31±0.92, P=0.04) as well as the L/A ratio (5.53±6.05 vs. 2.40±2.35, P=0.02) were found to be significantly higher in the women with MS compared to the healthy women.
Conclusion: The results from this study contribute to understanding that women with MS carry a higher atherogenic risk. The plasma L/A ratio has been proposed as a preferential marker of atherosclerosis susceptibility compared to leptin and adiponectin alone.

Keywords: Atherogenic index of plasma, Castelli risk index I and II, Leptin/adiponectin ratio, metabolic syndrome

1. Introduction

Metabolic syndrome (MS) refers to the clustering of cardiovascular risk factors that include type 2 diabetes mellitus (DM), obesity, dyslipidaemia and hypertension (1, 2). It has been estimated that people with MS have double the risk of developing cardiovascular disease (CVD) and experience a five-fold increased risk of type 2 diabetes mellitus (DM) compared with those without the syndrome (1, 3).

Dyslipidemia has been identified as one of the most important risk factors associated with coronary artery disease (CAD) (4). Low HDL-cholesterol (HDL-C), high triglycerides (TG) and high LDL-cholesterol (LDL-C) levels have been associated with increased incidence of CAD (5). It has been suggested that the different combinations of these lipid profile parameters can be used to identify such high risk individuals. Atherogenic Index of Plasma (AIP) and Castelli Risk Indexes (CRI) are the ratios that have been studied as markers of lipid atherogenic risk. These are the calculated fractions which can be used in the clinical setting for assessing the risk of CVD beyond the routinely done lipid profile.

Although adiposity has been traditionally defined as an increase in total body mass, visceral fat accumulation has been found to correlate with a cluster of metabolic abnormalities observed among the patients with MS (6). Visceral adipose tissue contains large insulin-resistant adipocytes; it has a well-developed vascular system and is characterized by infiltration of a large number of inflammatory cells. Inflammatory cells regulate adipocyte behaviour as a source of hormones and cytokines, called adipokines, with proinflammatory and proatherogenic effects. Circulating levels of cytokines including resistin, leptin, TNFa, interleukin-6 (IL-6), C-reactive protein (CRP), fibrinogen and plasminogen activator inhibitor-1 (PAI-1) are generally increased in obese subjects and in patients with diabetes mellitus. On the contrary, visceral adiposity is a state with a relative deficiency of adiponectin, a

tissue-specific circulating hormone with insulin-sensitising and anti-atherogenic properties. Adiponectin stimulates glucose use and fatty acid oxidation in the muscle, enhances insulin sensitivity in the liver, increases free fatty acid (FFA) oxidation, reduces hepatic glucose output and inhibits monocyte adhesion and macrophage transformation to foam cells within the vascular wall (7-9). Therefore, it appears that the presence of leptin-adiponectin (L/A) imbalance may be at great importance for the elevated risk of developing type 2 DM and CVD associated with abdominal obesity (10).

The *aim* of the study was to evaluate the persisting cardiovascular risk in women with insulin resistance syndromes using AIP, CRI I and II, and the L/A ratio.

2. Materials and methods:

The present study was conducted in the Clinic of Endocrinology and metabolic diseases at "Sveti Georgy" University Hospital, Plovdiv. It comprised of 31 women with MS and 30 clinically healthy women forming the control group. The following clinical measurements and laboratory tests were performed in all participants: body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressure (SBP and DBP), fasting plasma glucose (FPG), fasting immunoreactive insulin (IRI), total cholesterol (TC), HDL-C, TG, leptin and adiponectin. Body mass index (BMI), waist-hip ratio (WHR), homeostasis model assessment insulin resistance index (HOMA index), LDL-C, AIP, Castelli' s I and II risk indexes and the L/A ratio were calculated.

Waist circumference was defined in the horizontal plane midway between the lower edge of the 12th rib and the top of the iliac crests. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor. LDL-C was calculated according to Friedewald formula: {TC – (HDL-C + TG/2.2)}. SBP and DBP were measured in a sitting position after at least 5 minutes' rest. All blood samples were taken after an overnight 12-hour fast. The biochemical tests were performed under standard conditions in the central Clinical Laboratory at "Sveti Georgy" University Hospital, Plovdiv. Leptin and adiponectin levels were determined in the Clinical Centre of Endocrinology, "Acad. Ivan Pentchev" University Hospital of Endocrinology, Medical University, Sofia. All the study participants signed an informed consent and the study was approved by the local Ethics committee at "Sveti Georgy" University Hospital, Plovdiv.

The diagnosis of MS was made according to the criteria of the International Diabetes Federation (IDF), when central obesity as a mandatory component was present (waist circumference ≥80 cm for Caucasian women) in combination with at least two of the following abnormalities: elevated TG >1.7 mmol/l; decreased HDL-C <1.29 mmol/l or specific treatment; elevated blood pressure ≥130/85 mmHg or treatment of already diagnosed hypertension; increased FPG ≥5.6 mmol/l or diagnosed type 2 DM. A comprehensive set of hormonal tests was done in all study participants (androgens, gonadotropins, oestradiol, prolactin, TSH, free T4, serum cortisol at 8 h and 22 h) for diagnostic purposes and in order to exclude pregnancy and/or other endocrine pathology: Cushing's syndrome, inherited adrenal hyperplasia, prolactinoma, hypo-/hyperthyroidism, hypopituitarism, hypogonadism, polycystic ovary syndrome etc. Venous blood samples for the determination of the hormonal parameters were collected in the early morning hours after a 12-hour fast. Insulin was tested using a commercial kit for quantitative determination of immunoreactive insulin on the basis of microparticular immunoenzyme analysis (MEIA) on an AxSYM system (ABBOTT, USA) with the following characteristics: sensitivity ≤0.8 mIU/ml; inter-assay variation, CV% <2.9; intra-assay variation CV%<5.3. TC was determined by ChOD, PAP; TG by GPO, PAP, and HDL-C by MgSO4-dextran SO4 precipitation, Schneiders Analysers; Netherlands test; Delta Kone Autoanalyser. Serum glucose levels were determined by a standard GOD-POD method. The hormonal tests were performed on an Ax-SYMTM System (Abbott Diagnostics, Abbott Park, USA) using commercial kits. *Leptin* was determined by means of a solid-phase immunoenzyme assay (Human) ELISA (commercial kit of DRG, Germany) with the following characteristics: sensitivity, 0.2 ng/ml; inter assay variation, CV% < .4; intra assay variation, CV% < 8.7%; correlation with RIA, r = 0.95. Serum levels of adiponectin were tested by ELISA (Human Adiponectin Elisa, commercial kit of BioVendor) with: sensitivity - 26 ng/ml; inter assay variation - CV% < 7.0; intra assay variation - CV% <5.9.

In all participants BMI (weight (kg)/height (m)²), WHR (waist (cm)/hip(cm)), homeostasis model of insulin resistance index (HOMA-IR) (fasting blood glucose (mmol/l) x IRI (μIU/ml)/22,5), atherogenic index of plasma (AIP) {(logTG)/HDL-C} , Castelli's risk index I and II (TC/HDL-C and LDL-C/HDL-C, respectively) and the L/A ratio were calculated. A cut-off of 2 for HOMA-IR was used for determining the presence of IR.

The statistical analysis was performed by SPSS version 21.0. The results are presented as mean ± standard deviation (SD). For comparison of variables with a normal distribution unpaired, 2-tailed Student's t-test was used, whereas the Mann–Whitney U-test was used for variables with a skewed distribution to analyse clinical and laboratory data. Statistical significance was taken at level of $P<0.05$.

3. Results:

The clinical and metabolic characteristics of the studied women (anthropometric parameters;values of glucose and lipid metabolism; SBP and DBP) are presented in Tables 1 and 2. Table 3 shows the results of the calculated atherogenic ratios: AIP, Castelli's risk indexes I and II and the L/A ratio. There were no statistically significant differences between the groups in terms of age, WHR, FPG, TC, LDL-C or adiponectin. The women with MS had significantly higher weight, BMI, waist circumference, hip circumference, fasting IRI, HOMA index, SBP and DBP as well as significantly higher levels of TG and leptin compared to the control women. AIP (-0.006±0.32 vs. -0.212±0.26, P=0.008), Castelli' s risk indexes I (4.39±1.21 vs. 3.64±1.07, P=0.01) and II (2.82±1.01 vs. 2,31±0.92, P=0.04) and the L/A ratio (5.53±6.05 vs. 2.40±2.35, P=0.02) were found to be significantly higher in the women with MS compared to the healthy women.

Table 1. Clinical and anthropometric characteristics of the studied groups.

Parameters	**Groups of women**	
	Control women (n=30)	**Women with MS (n=31)**

Age (years)	26.33±4.49	29.03±6.16
Weight (kg)	61.71±10.21	98.40±13.94***
BMI (kg/m²)	22.58±2.84	34.98±5.71***
Waist (cm)	78.05±8.03	105.41±7.96***
Hip (cm)	98.61±10.22	125.38±11.64***
WHR	0.80±0.08	0.83±0.06

* P<0.05; ** P<0.01;*** P<0.001.

Table 2. Metabolic parameters, SBP, and DBP in the studied groups.

Parameters	**Groups of women**	
	Control women (n=30)	**Women with MS (n=31)**
GLU 0' (mmol/l)	4.79±0.55	5.06 ±0.68
IRI 0' (µIU/ml)	6.37±2.51	10.67±4.01***
HOMA-IR	1.39±0.65	2.45±1.08***
TC (mmol/l)	4.62±1.06	4.87±0.89
HDL-C (mmol/l)	1.35±0.41	1.16±0.26*
LDL-C (mmol/l)	2.87±0.97	3.09±0.75
TG (mmol/l)	0.87±0.38	1.35±0.79**
SBP (mmHg)	111.35±11.25	122.10±8.44***
DBP (mmHg)	71.35±8.70	77.26±9.82*

* P<0.05; ** P<0.01;*** P<0.001.

Table 3. Hormonal parameters and lipid atherogenic risk indexes in the studied groups.

Parameters	**Groups of women**	
	Control women (n=30)	**Women with MS (n=31)**
Leptin (ng/ml)	22.47±14.40	50.28±18.46***
Adiponectin (µg/ml)	14.28±8.62	13.23±7.38
L/A ratio	2.40±2.35	5.54±6.05*
AIP	- 0.21±0.26	- 0.01±0.33**
CRI I	3.64±1.07	4.39±1.21*
CRI II	2.31±0.92	2.82±1.01*

* P<0.05; ** P<0.01;*** P<0.001.

4. Discussion:

In individuals with type 2 DM and MS, cardiovascular risk is increased by a clustering of risk factors such as abdominal obesity, impaired fasting glucose, increased blood pressure, low HDL-C, increased TG, and an increase in small, dense LDL particles. The current increase in the incidence of type 2 DM in the population perhaps poses the most urgent cardiovascular risk (11). CAD is the epidemic of modern civilization in which dyslipidemia contributes significantly to its pathogenesis. WHO predicts 11.1 million deaths globally and 71% deaths in developing countries due to CAD by 2020 A.D.

Lipid profile refers to some routinely done biochemical tests to assess the atherogenic status of individuals at risk of CAD. It includes serum TG, serum TC and its sub fractions like HDL-C and LDL-C. The Framingham heart study over years has established the role of deranged lipid profile in the progression of CAD and deranged LDL-C levels are the primary target for treatment (12). Calculating certain ratios using these parameters, especially in situations where LDL-C levels are below target range, may increase the identification of at-risk individuals.

A number of lipid related parameters have been used to predict the risk of CAD. According to Grover, either the ratio of LDL-C/HDL-C or TGL /HDL-C is the best related predictor of future cardiovascular events (13). The logarithmically transformed ratio of plasma TG to HDL-C correlates closely with the LDL particle size and can serve as an indicator of the atherogenic lipoprotein phenotype. Clinical studies have shown that AIP predicts cardiovascular risk and that it is an easily available cardiovascular risk marker as well as a useful measure of the response to treatment (14). Patients with type 2 DM are at increased risk of cardiovascular morbidity and mortality. The significant increase in TG, the TGL/HDL-C ratio, AIP, BP and the BMI of diabetic patients with MS when compared to the type 2 diabetic patients without MS, predicts that patients with type 2 DM with MS are at a higher risk level for CVD. This is in agreement with the fact that insulin resistance induces several metabolic changes such as hyperglycaemia, dyslipidaemia and perhaps to a lesser extent, hypertension, which all contribute to the development of atherosclerosis (15).

This study aimed at evaluating the role and contribution of the ratios like AIP and CRI I and II in lipid atherogenic risk, respectively CAD. In the current study we found that AIP was significantly higher in the MS women as compared to the controls (-0.006±0.32 vs. -0.212±0.26, P=0.008). Studies have shown an inverse relationship existing between TG and HDL-C and that the ratio of TG to HDL-C is a strong predictor of infarction (16). AIP is being used by some practitioners as a significant predictor of atherosclerosis. It has been suggested that AIP values of -0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high cardiovascular risk (17). We observed an AIP ratio of - 0.006 in women with MS and - 0.212 in controls which are in concordance with the suggested cut-offs. Moreover, some studies have shown that in situations where other atherogenic risk parameters like TG and HDL-C appear normal, AIP may be a diagnostic alternative (18). Studies have shown its role in predicting cardiovascular risk and effectiveness of therapy (19).

Castelli's Risk indexes (CRI) are based on three important lipid profile parameters i.e. TC, LDL-C and HDL-C. CRI-I calculated as the ratio of {TC/HDL-C} and CRI-II as {LDL-C/HDL-C} (20), were found to be significantly higher in women with MS compared to the controls (4.39±1.21 vs. 3.64±1.07). In our study we did not find a significant difference in TC and LDL-C levels between the two studied groups, whereas the ratios based on these parameters showed

significant differences between the groups. This clearly suggests the relevance of ratios over individual lipid parameters especially in situations where the drug management might be affected. The Canadian working group had chosen the TC/HDL-C ratio as a secondary goal of therapy considering it to be a more sensitive and specific index of cardiovascular risk than TC, particularly in individuals with TG>3.39 mmol/l (21). We found CRI I > 4 in our pathological group which is in concordance with other studies (22). Some studies have shown an association of TC/HDL-C ratio with coronary plaques formation (23). In our women with MS CRI-II (2.82±1.01) was found to be higher than in the healthy women and close to the upper limit for normal range, that is 3. In PROCAM study, it was observed that subjects with LDL-C/HDL-C >5 had a 6-time higher rate of coronary events (24).

Essiarab et al. studied the impact of obesity and MS on lipoprotein profiles and cardiovascular risk through lipid ratios and AIP in Moroccan women. Their study included 240 Moroccan women divided into 3 groups: controls (Group 1), obese women without MS (Group 2) and obese women with MS (Group 3). Anthropometric and lipid measurements were taken and specific lipid ratios assessed, as well as non-HDL cholesterol (Non-HDL-C) and AIP. Group 2 presented similar lipoprotein profiles compared with Group 1. Group 3 had higher TG levels than Group 1, which, in turn, increased HDL and AIP values. Dyslipidemia in Group 3 was demonstrated by higher TG levels, lipid ratios and AIP and lower HDL-C levels compared with Group 2. All of these abnormalities are responsible for elevation of CVD risk. Closer associations were found between cardiovascular risk and lipid ratios and AIP than lipids alone. The study confirmed that MS affects the serum lipoprotein profile of obese women. Lipid ratios, non-HDL-C and AIP remained useful tools for the diagnosis and prognosis of CVD by their associations with lipid parameters and their high predictive values (25)**.**

It is well known that obesity is associated with increased cardiovascular morbidity, mortality and metabolic derangements including insulin resistance, dyslipidemia, and hypertension (26-28). Although the mechanisms linking obesity and CVD are not completely understood, recent evidence indicates that inflammation and insulin resistance may play important roles. It has been recognized in recent years that adipose tissue is an important endocrine organ, secreting several bioactive molecules termed adipokines, which regulate whole-body metabolism and the immune response. Leptin and adiponectin, the two best-characterized adipokines, respond in a reciprocal manner to increasing adiposity. Plasma levels of leptin are elevated in obese individuals, and increased levels of leptin have been associated with higher levels of CRP (29) and are predictive of future cardiovascular events (30). In contrast, plasma adiponectin levels are reduced in obese individuals, and lower levels of adiponectin are associated with higher levels of CRP (31) and correlate significantly and independently with CAD. Two recent studies have demonstrated that vascular remodeling and neointimal formation are markedly attenuated in leptin deficient *ob/ob* mice and *db/db* mice with leptin receptor mutation (32, 33), suggesting that leptin may accelerate the development of vascular injury. Conversely, studies with adiponectin-deficient mice have revealed that adiponectin plays a protective role in the development of atherosclerosis (34). Mounting evidence also suggests that hyperleptinemia and hypoadiponectinemia are associated with insulin resistance (35), and the L/A ratio correlates with insulin resistance better than either leptin or adiponectin levels alone. The L/A ratio is a powerful independent predictor of intima media thickness (IMT) in healthy subjects and correlates with several anthropometric, metabolic, and clinical parameters better than each single adipokine (36).

Calculating the L/A ratio in our studied groups of women, we found significantly higher values of the ratio in the women with MS compared to the controls (5.53±6.05 vs. 2.40±2.35, P=0.02).

A study of Kazuhiko Kotani and Naoki Sakane (37) suggested that the L/A ratio can serve as a clinically useful marker for detecting MS characteristics in the general Japanese population. Japanese subjects (n=678 - 208 men and 470 women, mean age 58.8±14.4 years) took part in the study. Biochemical markers such as leptin and adiponectin present were measured. The results showed that the L/A ratio in subjects with MS was significantly higher than that in subjects without MS, regardless of gender. The L/A ratio also showed a significant and gradual increase corresponding to the increase in the number of components of MS present in both the genders (P<0.01). The cut-off level of the L/A to detect MS was 0.59 (sensitivity: 0.72, specificity: 0.70) in men and 1.04 (sensitivity: 0.72, specificity: 0.69) in women (37).

In conclusion, our study is in conformity with the huge source of evidence that lipid ratios like Atherogenic Index of Plasma, Castelli risk indexes and the L/A ratio could be used for identifying individuals at higher risk of cardiovascular disease in the clinical setting especially when the absolute values of individual lipoproteins seem normal. The aforementioned observations confirm that insulin resistance and existing metabolic syndrome can contribute to a prothrombotic and proatherogenic state leading to the development of cardiovascular diseases.

References

[1] S.M. Grundy, "Metabolic syndrome pandemic", *Arterioscler Thromb Vasc Biol*, 28, pp 629-636, 2008.

[2] A.J. Cameron, J.E. Shaw, P.Z. Zimmet, "The metabolic syndrome: prevalence in worldwide populations", *Endocrinol Metab Clin North*, 33, pp 351-375, 2006

[3] R.W. Nesto, "The relation of insulin resistance syndromes to risk of cardiovascular disease", *Rev Cardiovasc Med* , 4, pp S11-S18, 2003

[4] S. Yusuf, S. Hawken, S. Ounpuu, T. Dans, A. Avezum , F. Lanas, M. McQueen, A. Budaj, P. Pais, J. Varigos, J. Lisheng, "Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study". Lancet, 364 (9438), pp 937-952, 2004.

[5] D. Weissglas-Volkov and P. Pajukanta, "Genetic causes of high and low serum HDL cholesterol", The Journal of Lipid Research, 51, pp 2032-2057, 2010.

[6] J.P. Despres, "Health consequences of visceral adiposity", Ann Med, 33, pp 534-541, 2001.

[7] J.G. Nells, J.M. Olefsky, "Inflamed fat: what starts the fire?", *J Clin Invest*, 116, pp 33-35, 2006.

[8] P. Trayhum , I.S. Woods, "Adipokines: inflammation and the pleiotropic role of white adipose tissue", *Br J Nutr* , 92, pp 347-355, 2004.

[9] D.I. Koleva, M.M. Orbetzova, P.K. Atanassova, "Adipose Tissue Hormones and Appetite and Body weight Regulators in Insulin Resistance", Folia Medica Journal, 55(1), pp 25-32, 2013.

[10] P. Lopez-Jaramillo, D. Gomez-Arbelaez, J. Lopez-Lopez, C. Lopez-Lopez, J. Martinez-Ortega et al., "The role of leptin/adiponectin ratio in metabolic syndrome and diabetes", Horm Mol Biol Clin Investig, 18(1), pp 37-45, 2014.

[11] *L. Gilling, P. Suwattee, C.DeSouza, S. Asnani, V. Fonseca, "Effects of the* thiazolidinediones on vascular risk factors", Am J Cardiovasc Drugs, 2, pp 149–156, 2002.

[12] National Cholesterol Education Program (NCEP) Expert Panel on Detection,Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP)Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report, Circulation, 106, pp 3143–3421, 2002.

[13] S.A. Grover, C. Levington and S. "Panquet, Identifying adults at low risk for significant hyperlipidemia: a validated clinical index", *J Clin Epidemiol*, 52, pp 49-55, 1999.

[14] J. Frohlich, M. Dobiášová, "Fractional esterification rate of cholesterol and ratio of triglycerides to HDL-cholesterol are powerful predictors of positive findings on coronary angiography", *Clin Chem* , 49, pp 1873-1880, 2003.

[15] P. Kalidhas, D. Kanniyappan, K. Gandhi, R.M. Aruna, "Coronary Artery Disease Risk Factors in Type 2 Diabetes Mellitus with Metabolic Syndrome in the Urban South Indian PopulationJournal of Clinical and Diagnostic Research", 5(3), pp 516-518, 2003.

[16] J.M. Gaziano, C.H. Henne kens, C.J. O.Donnell, J.L. Breslow, J.E. Buring, "Fasting triglycerides, high density lipoprotein, and risk of myocardial infarction", Circulation, 96, pp 2520-2525, 1997.

[17] M. Dobiasova, "AIP-atherogenic index of plasma as a significant predictor of cardiovascular risk: from research to practice", Vnitr Lek, 52(1): pp 64-71, 2006.

[18] U.I. Nwagha E.J. Ikekpeazu, F.E. Ejezie, E.E. Neboh, I.C. Maduka, "Atherogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu, Nigeria", African Health Sciences, 10(3), pp 248–252, 2010.

[19] M. Dobiášová, J. Frohlich, M. Šedová, M.C. Cheung, B.G. Brown, "Cholesterol esterifi cation and atherogenic index of plasma correlate with lipoprotein size and findings on coronary angiography", J Lipid Res, 52, pp 566–571, 2011.

[20] W.P. Castelli, R.D. Abbott, P.M. McNamara, "Summary estimates of cholesterol used to predict coronary heart disease", Circulation, 67(4), pp 730-734, 1983.

[21] J. Genest, J. Frohlich, G. Fodor, R. McPherson, "The Working Group on Hypercholesterolemia and Other Dyslipidemias. Recommendations for the management of dyslipidemia and the prevention of cardiovascular disease", CMAJ, 169, pp 921–924, 2003.

[22] J. Subia, S. Afshan, "Comparison of CVD Risk Associated With the long Term use of Contraceptives In Young Females" , J App Pharm Sci, 2 (11), pp 062-066, 2012.

[23] D. Nair, T.P. Carrigan, R.J. Curtin, Z.B. Popovic, S. Kuzmiak, P. Schoenhagen, S.D. Flamm, M.Y. Desai, "Association of total cholesterol/ high-density lipoprotein cholesterol ratio with proximal coronary atherosclerosis detected by multislice computed tomography", Prev Cardiol, 12(1), pp 19-26, 2009.

[24] G. Assmann, P. Cullen, H. Schulte, "The Munster Heart Study (PROCAM). Results of follow-up at 8 years" Eur Heart J, 19, pp A2–A11, 1998.

[25] F. Essiarab, H. Taki, H. Lebrazi, M. Sabri, R. Saý¨le, "Usefulness of lipid ratios and atherogenic index of plasma in obese Moroccan women with or without metabolic syndrome", Ethn Dis., 24[2], pp 207–212, 2014.

[26] H.E. Lebovitz, M.A. Banerji, "Point: visceral adiposity is causally related to insulin resistance", *Diabetes Care*, 28, pp 2322-2325, 2005.

[27] A. Aneja, F. El-Atat, S.I. McFarlane, J.R. Sowers, "Hypertension and obesity", *Recent Prog Horm Res*, 59, pp 169-205, 2004.

[28] M.C. Carr, J.D. Brunzell, "Abdominal obesity and dyslipidemia in the metabolic syndrome: importance of type 2 diabetes and familial combined hyperlipidemia in coronary artery disease risk", *J Clin Endocrinol Metab*, 89, pp 2601-2607, 2004.

[29] B.G. Ble A,Windham, S. Bandinelli, et al, "Relation of plasma leptin to C-reactive protein in older adults", *Am J Cardiol*, 96, pp 991-995, 2005.

[30] A.D. Wallace AM,McMahon, C.J. Packard, et al, "Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS)", *Circulation*, 104, pp 3052-3056, 2001.

[31] K. Matsushita, H. Yatsuya, K. Tamakoshi, et al, "Inverse association between adiponectin and C-reactive protein in substantially healthy Japanese men", *Atherosclerosis*, 188, pp 184-189, 2006.

[32] K. Stephenson, J. Tunstead, A. Tsai, R. Gordon, S. Henderson, H.M. Dansky, "Neointimal formation after endovascular arterial injury is markedly attenuated in db/db mice", *Arterioscler Thromb Vasc Biol*, 23, pp 2027–2033, 2004.

[33] K. Scha¨fer, M. Halle, C. Goeschen, Dellas, M. Pynn, D.J. Loskutoff, S. Konstantinides, "Leptin promotes vascular remodeling and neointimal growth in mice", *Arterioscler Thromb Vasc Biol, 24, pp 112–117, 2004.*

[34] M. Matsuda, I. Shimomura, M. Sata, Y. Arita, M. Nishida, N. Maeda, M. Kumada, Y. Okamoto, H. Nagaretani, H. Nishizawa, K. Kishida, R. Komuro, N. Ouchi, S. Kihara, R. Nagai, T. Funahashi, Y. Matsuzawa, "Role of adiponectin in preventing vascular stenosis: the missing link of adipo-vascular axis", J Biol Chem , 277, pp 37487–37491, 2002.

[35] F.M. Finucane, N.J. Luan J,Wareham et al, "Correlation of the leptin: adiponectin ratio with measures of insulin resistance in nondiabetic individuals", *Diabetologia*, 52, pp 2345-2349, 2009.

[36] G.D. Norata, S. Raselli, L. Grigore, K. Garlaschelli, E. Dozio, P. Magni, A.L. Catapano "Leptin:Adiponectin Ratio Is an Independent Predictor of Intima Media Thickness of the Common Carotid Artery", Stroke, 38, pp 2844-2846, 2007.

[37] K. Kotani and N. Sakane, "Leptin:Adiponectin Ratio and Metabolic Syndrome in the General Japanese Population", Korean J Lab Med, 31, pp 162-166, 2011.

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Thesis Title: Adipose tissue hormones, regulators of appetite and body weight and markers of endothelial dysfunction in insulin resistance **Advisor:** Prof. Maria M. Orbetzova, MD, PhD
Since 2012 till now - A resident, Clinic of Endocrinology and Metabolic Diseases, University Hospital
"Sveti Georgy", Plovdiv, Bulgaria
Work experience and professional activities:
September 2007 - Professional exchange organized by International Federation of Medical Students' Associations, a clerkship in
Department of Internal Medicine, Policlinico G.B. Rossi, Verona, Italy
2010 - Medical training in Department of Palliative Medicine, Plovdiv
October 2010 - April 2011 - Medical practice in Department of Internal Medicine, Division of Cardiology, "Sveti Mina" Hospital, Plovdiv
17-19th June 2011 – Active participation in the 3rd International Accu-Chek Diabetes management and Education Conference, Sofia, Bulgaria
21.09-27.09.2014 - Open Medical Institute Seminar in Salzburg – International Atherosclerosis Society Seminar in Lipid Metabolism – participation with a case report
26-28 March 2015 - 3rd EASD Postgraduate Course on Clinical Diabetes and its Complications, Sofia, Bulgaria
16-18 July 2015 - Sinaia, Neurodiab Summer School – Autonomic neuropathy. From academic knowledge to clinical practice
2011-2014 – a participant in 2 projects of Medical University of Plovdiv in the field of metabolic syndrome
Membership in scientific societies: Bulgarian Society of Endocrinology – Management Board
Scientific interests and publications in the field of: , obesity and appetite regulator hormones , adipocytokines and insulin resistance , metabolic syndrome, PCOS, endothelial dysfunction and cardiovascular diseases

Second author profile

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Education:
2004-2009 – Deapartment of Pharmacology and Toxicology, Medical University – Sofia, 2, Zdrave str. Specialization in Pharmacology
1998-2003 – Central Clinical Laboratory, MHAT "Tzariza Joanna", Medical University – Sofia, 8, Bialo more str. PhD
1993-1997 Medical faculty, Medical University – Sofia, Public Health specialization
1986-1992 Faculty of Medicine, Medical University – Sofia,
1981-1986 Lycee de la langue francaise Sofia Diplome № 016113 / 04.07.1986
Previous Experience for the last 5 years (Organization, Position, Title, Dates):
Since 2010 till now Chef Administrative Assistant, Medical University-Sofia, Preclinical Education Center, Department of Pharmacology and Toxicology, 2, Zdrave str.
Since 2010 till now Chef Assistant, Sofia University, Medical Faculty, Department of Internal Medicine, Pharmacology and Clinical Pharmacology, Pediatrics, Epidemiology, Infectious Diseases and Dermatology
Major Fields of Scientific Research:
Pharmacology, Toxicology, Metabolism
Membership in Scientific and/or Professional Institutions, Bodies, and Organizations:
Bulgarian Association for Study of Obesity and Related Diseases (BASORD)
Bulgarian Scientific Association of Pharmacology
Bulgarian Scientific Association of Toxicology
Bulgarian Association of Clinical Researches (BACR)

Third author profile

Professor Maria Mitkova Orbetzova, MD, PhD
Head of Clinic of Endocrinology and Metabolic Diseases, "Sveti Georgy" University Hospital, Medical University - Plovdiv, Bulgaria
Relevant Education:
1986 - Higher education, Specialty in Medicine, Higher Medical Institute, Medical Academy, Medical University - Sofia;
1991 - Specialty Internal Medicine, Higher Medical Institute, Medical University - Sofia;
1993 - Specialty Endocrinology and Metabolic diseases, Higher Medical Institute, Medical University - Sofia;
2005 - Academic degree Associate Professor, Medical University – Sofia;
2011 - Master degree in Public Health and Health Management, Medical University – Sofia;

2013 - Academic degree Professor, Medical University – Plovdiv;

Relevant Previous Positions:

1987-2005 - Senior Research Fellow, Assistant Professor, Clinical Centre of Endocrinology and Gerontology, ″Acad. Iv. Penchev″ University Hospital, Medical University – Sofia

1986-1987 – Intern, Department of Internal Medicine with intensive sector, Primary Regional Hospital – Byala Slatina; "Pioner" and "9th Septemvri" Factories, Byala Slatina, Municipality of Montana, Bulgaria.

Relevant Job Related Training:

1993 - Specialization (IDF scholarship), Clinic of Endocrinology and Diabetes, Medical School, Department Medicine, University New Castle, England;

1997 - Course "Practical Diabetology", Steno Diabetes Centre, Gentofte and Copenhagen, Denmark;

1999 - Specialization, Clinic of Arterial Hypertension, "Brousse" Hospital, Paris, France;

2009 - Training course for treatment of diabetic foot, Diabetic Foot Clinic, King's College Hospital, London;

2012 - Qualification course in the field of diabetes, University "St. Andrew", United Kingdom.

Other Activities Pertinent to Professional Qualifications

Project Manager in 4 and collaborator in 6 Projects of Ministry of Education and Science; Project Manager in 12 Projects of Medical University-Sofia and Medical University-Plovdiv, Bulgaria

Collaborator in MEDUCATOR- an International Project of Medical University-Plovdiv – E-learning in medicine

Supervisor of 8 PhD students, consultant of 4 PhD students, Supervisor of 15 specializations in Endocrinology;

Member of the Ethics Committees at "Akad. Iv. Penchev" University Hospital - Sofia (former) and "Sveti Georgy" University Hospital - Plovdiv

Vice Dean "Education", Faculty of Medicine and Member of the Academic Council of Medical University-Plovdiv

Editor-in-Chief of "ENDOCRINOLOGIA" Journal - printed edition of the Bulgarian Society of Endocrinology

Membership in scientific societies:

Bulgarian Society of Endocrinology – Management Board

Bulgarian Institute of Metabolic Syndrome – Management Board

Bulgarian Association of Endocrine Hypertension /Alliance/- Management Board

European Society of Endocrinology

European Association of Gynecological Endocrinology

European Association for the Study of Diabetes and Diabetes Education Study Group

European Thyroid Association

Scientific interests and publications in the field of:

PCOS, reproductive disorders and hyperandrogenic states in women; insulin resistance, metabolic syndrome, obesity, adipocytokines and appetite regulator hormones, diabetes mellitus; Cushing's syndrome; endocrine hypertension; pituitary tumours; osteoporosis.